PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference REP07190WO	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 03/04104	International filing date *(day/month/year)* 26.09.2003	Priority date *(day/month/year)* 27.09.2002
International Patent Classification (IPC) or both national classification and IPC C12N9/88		
Applicant PANTHERIX LTD. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
16.04.2004	19.11.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Vix, O Telephone No. +49 89 2399-7326

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/04104

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-25 as originally filed

Claims, Numbers

1-9 as originally filed

Drawings, Sheets

1-189 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1-5

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-5

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	6-9
	No:	Claims	
Inventive step (IS)	Yes:	Claims	6-9
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	6-9
	No:	Claims	

2. Citations and explanations

see separate sheet

Reference is made to the following documents:

D1: SCHONBRUNN ERNST ET AL: "Interaction of the herbicide glyphosate with its target enzyme 5-enolpyruvylshikimate 3-phosphate synthase in atomic detail" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 98, no. 4, 13 February 2001, pages 1376-1380

D2: MACHEROUX PETER ET AL: "Evidence for a major structural change in Escherichia coli chorismate synthase induced by flavin and substrate binding" BIOCHEMICAL JOURNAL, vol. 335, no. 2, 15 October 1998, pages 319-327,

D3: ABAGYAN R ET AL: "HIGH-THROUGHPUT DOCKING FOR LEAD GENERATION" CURRENT OPINION IN CHEMICAL BIOLOGY, CURRENT BIOLOGY LTD, LONDON, GB, vol. 5, no. 4, August 2001, pages 375-382,

D4: GB-A-2 374 414 (PANTHERIX LTD) 16 October 2002 (2002-10-16)

D5: AHN HYUNG JUN ET AL: "Crystallization and preliminary X-ray crystallographic studies of chorismate synthase from Helicobacter pylori." ACTA CRYSTALLOGRAPHICA SECTION D BIOLOGICAL CRYSTALLOGRAPHY, vol. 59, no. 3, March 2003, pages 569-571,

D6: ROBERTA F ET AL: "EVIDENCE FOR THE SHIKIMATE PATHWAY IN APICOMPLEXAN PARASITES" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB, vol. 393, no. 6687, 26 June 1998, pages 801-805,

Re Item III

Non establishment of opinion with regard to novelty, inventive step or industrial applicability

The applicant is advised that the examination will be restricted to the searched part. As mentioned in the ISR, no meaningful search could be carried out for the subject-matter of claims 1-5. Therefore, no meaningful opinion with respect to novelty, inventive step or industrial applicatibility of claims 1-5 will be given.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Novelty (Art. 33(2) PCT)

The application relates to the crystal structure of chorismate synthase (also known as 5-enolpyruvylshikimate 3-phosphate phospholyase) from Streptococcus pneumoniae and identification of the critical residues of the binding domain.

1.1 The shikimate pathway is known to be an attractive target for herbicides and antimicrobial agents because it is essential in algae, higher plants, bacteria and fungi, but absent from mammals (see D6).

D1 relates to the identification of the interaction of 5-enolpyruvylshikimate 3-phosphate synthase (EPSP synthase) from E. coli (EC 2.5.1.19) with one of its two substrates (shikimate 3-phosphate) and with the widely used herbicide glyphosate by X-ray crystallography (see PDB structure accession codes 1G6S and 1G6T). This is an enzyme structure of an enzyme involved in the earlier pathway of chorismate as it produces EPSP, a precursor used by the chorismate synthase to produce chorismate. In consequence, the structure in D1 appears to be distinct from the present one which refers to chorismate synthase (or 5-enolpyruvylshikimate 3-phosphate phospholyase: an enzyme which uses EPSP to produce chorismate). Therefore, it appears that no previous chorismate synthase X-ray structure has been disclosed in the prior art.
Thus, as long as the subject-matter of the claims is limited to the solved chorismate synthase X-ray structure from Streptococcus pneumoniae disclosed in the application and methods using this chorismate synthase 3D structure coordinates, or one of its domain, the subject-matter of claims 6-9 appears to be novel in view of the available prior art.

1.2 However, D5 discloses a chorismate synthase (EC 4.6.1.4) from Helicobacter pylori fused with an eight-residue C-terminal tag which was overexpressed in soluble form in Escherichia coli and crystallized at 296 K using PEG 400 as a precipitant. A set of X-ray diffraction data was collected to 2.5 Å resolution using synchrotron radiation. In the case of a non valid priority, D5 might become relevant for novelty due to the "root mean square deviation of the conserved backbone residues" wording used in the claims: when aligning the backbone atoms of both structures, the rmsd is 1 Å over the 316 residues (using the secondary structure matching tool provided by the

Macromolecular Structure Database group at EBI).

2. Inventive step (Art. 33(3) PCT)

2.1 D2 discloses the chorismate synthase (EC 4.6.1.4) enzyme which catalyses the conversion of 5-enolpyruvylshikimate 3-phosphate (EPSP) into chorismate, and requires reduced FMN as a cofactor. It is shown in D2 that the binding of oxidized FMN and EPSP to chorismate synthase affects the properties and structure of the protein. This structural study involves techniques such as CD-spectroscopy, stopped-flow spectrofluorometry, FT-IR spectroscopy as well as small angle X-ray scattering experiments.

The technical problem to be solved by the present invention may therefore be regarded as the provision of an alternative structural study of a chorismate synthase. The solution would be to provide a crystal of a chorismate synthase and to crystallise it in order to determine its crystal structure.

Using an alternative chorismate sequence identified from the available public sequence databases, it would be possible to the skilled person to overexpress an enzyme for crystallographic studies.

The skilled person in the art willing to crystallise this enzyme knows that protein crystallisation requires highly homogeneous protein at high concentration for crystallisation trials. Trials with different reservoir solution is trivial in the art of protein hanging drop protein crystallisation and the screening/obtention for chorismate synthase crystals would be within the easy reach for the skilled person as illustrated for another EPSP interacting enzyme in D1.

However, if macromolecule crystallisation (**using well known strategy** in the field of crystallisation such trials based on sparse matrix) has to be considered as inventive due to the **unpredictability of the art of growing crystals**, inventive step can only be acknowledged to the observed and disclosed surprising technical effect (e.g. specific crystal or method for obtention of a crystal that diffracts X-ray at high resolution) effectively obtained using specific macromolecules and crystallisation conditions.

Thus, as long as the subject-matter of the claims is limited to the solved chorismate synthase X-ray structure from Streptococcus pneumoniae disclosed in the application and methods using this chorismate synthase 3D structure coordinates, or one of its domain, the subject-matter of claims 6-9 appears to inventive in view of the available prior art.

Thus said claims do not meet the requirements of Article 33(3) PCT.

2.2 The Applicant's attention is drawn to the fact that claim 8 lacks clarity (Art. 6 PCT) because of step [c] which refers to a "synthesis" step of an hypothetical and undisclosed agent or inhibitor which conflicts with the claim category which clearly relates to an identification method: "method of identifying a ligand". Such a step [c] will not be allowed in the Regional phase. The same applies for step (d). An objection under Article 5 PCT also arises since the existence of such compounds is merely speculative.